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Green synthesis and characterisation of ZnO nanoparticles from *Manihot esculenta* (cassava) peel and their antibacterial study

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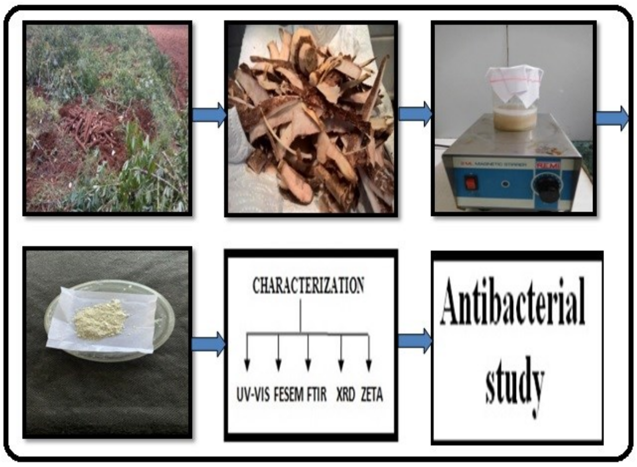
Graphical abstract



Abstract

A technique for green manufacturing of zinc oxide-based nanoparticles utilising cassava peel has been approached in this research for antibacterial investigation. Especially, the cassava peel is an agro waste from starch and food processing industries and the extract of Cassava peel is used for synthesising the zinc oxide nanoparticles (ZnO-NPs). The peel-based zinc oxide nanoparticles were synthesized using bioreduction method. The plasmon peak from UV–Visible spectroscopy is observed at 380 nm, while the XRD shows an average crystallite size of 67.31nm with hexagonal structure and also the stability of bio synthesised nanoparticles is confirmed by FTIR study. Additionally, FESEM confirms the structure is found to be spherical whereas Zeta potential analysis has confirmed the nanoparticles have a stable and well-dispersed form. Furthermore, the anti bacterial study against bio synthesized ZnO-NPs is carried out for both gram positive and gram negative bacteria. Therefore, the results proved that the bacteria are found to be more active against the synthesized nanoparticles.

Keywords: Cassava peel, green synthesis, *Manihot esculenta*, nanoparticles, ZnO

1. Introduction

Nanotechnology is the emerging technology for its size and morphology of the nanomaterials which possess large number of applications in the varied fields (Fakhari *et al.*, 2019). Nanoparticles can be of different dimensions; Zero, one, two and three depending on its shape. Researchers realized the importance of these nanoparticles when they determined that the size has an influence on physio-chemical properties of substances (Ijaz *et al.*, 2020). A zinc oxide-based nanoparticle attracts the attention of the researcher because of its morphology, antibacterial and antifungal activity towards more number of bacterial and fungal species (Jamdagni *et al.*, 2018). Due to the impact of bulk ZnO, arsenic and sulphur cannot be removed from water and wastewater. ZnO nanoparticles have been used to remove sulphur and arsenic from water because they have a bigger surface area than the bulk material (Sagar Raut and Thorat, 2015). ZnO nanoparticles were synthesized from both physical and chemical methods such as chemical reduction method (Guzmán *et al.*, 2009), solvothermal method (Cho *et al.*, 2009), sol-gel method (Yiamsawas *et al.*, 2009) and inert gas condensation method (Chang and Tsai, 2008). Moreover, the chemical methods require pernicious chemicals like methanol and methylbenzene resulting in environmental issues and toxic residues causing harm to human, environment and other organisms (Gao *et al.*, 2008). Also, green nanoparticle synthesis reduces the energy consumption and facilitates the removal of hazardous waste. As a result, a paradigm shift towards the bio-nano interaction has been witnessed by the researchers (Jan *et al.*, 2020; Rastogi *et al.*, 2018; Haseena *et al.*, 2022).

Plant products provide a platform for the biosynthesis of nanoparticles which is one of the biological resources. Plant-based biosynthesis has recently gained popularity as it is simple, affordable, and harmless (Ahmad et al., 2019; Raja et al., 2018). Since many organisms have become multidrug resistant, zinc oxide nanoparticles could replace antibiotics due to their numerous benefits and antibacterial properties (Bala et al., 2015). Zinc oxide, in particular has been extracted from a variety of plants, leaves, flowers and root extracts including *Anoectochilus* *elatus* (Vijayakumar et al., 2022), *Abutilon indicum, Clerodendrum infortunatum, Clerodendrum inerme* (Khan et al., 2018), *Moringa oleifera* (Matinise et al., 2017), *Hibiscus sabdariffa* (Soto-Robles et al., 2019) and *Furcraea foetida* (Sitrarasi et al., 2022), *Pelargonium odoratissimum* (Abdelbaky et al., 2022), *Plantain Peel extracts* (Imade et al., 2022) *have* proved a best source of Nanoparticles. Cassava is consumed as a source of calories in tropical countries like Africa, Asia, and Latin America, alongside rice and corn. Furthur, the root of the cassava plant contains starch which is highly digestible with important nutritional values. The products especially from cassava are the source of food for people over 500 million to 1 billion people in tropical countries. Global production of cassava ranges over 160 million tons per annum, and ranked 4th in crop's worldwide production after rice, wheat and maize (Murugan et al., 2012; Enwerem et al., 2020). In Tamilnadu, the Tapioca production is more in Salem, Erode, Namakkal districts. The annual production of Tapioca is around 38.81 lakh tonnes. Over 800 Sago industries are situated in and around the research area. The peel of the cassava remains as agro-wastes and untreated wastes leading to unsanitary condition. Therefore, the cassava waste is used in this study for the synthesis of ZnO-NPs. The biological method of synthesizing the nanoparticles over physical and chemical methods is low cost, eco-friendly, non-toxic, clean and can be easily used for large scale synthesis. The synthesised ZnO-NPs are characterised using UV-Visible Spectroscopy, Fourier Transform Infrared Spectroscopy, XRD, FESEM, Zeta potential and antibacterial study is carried out as well. Zinc Oxide nanoparticles are nontoxic in nature since they are semiconducting nanoparticles. The literatures have suggested that this is the first effort towards extraction of ZnO nanoparticles from Cassava peel.

2. Materials and methods

2.1. Collection and processing of cassava peel

The cassava peel collected from Jambai, Bhavani, Erode district, Tamilnadu, India with a latitude and longitude of 11° 27' 8.7624'' N, 77° 41' 35.7108'' E was used for the study. The fresh tubers were peeled out and washed in running water for several times to remove the dirt and soil. As the Cassava peel contains more lignin content, the peel was washed with water. The extracted peel (1 g) was further extracted for 3 hours using 100 ml of the 95% ethanol. The precipitates were washed several times with sterile distilled water and kept in the fume hood at room temperature (25 ± 2ºC) for 2 h before drying in a hot air oven at 60ºC for 24 h. The sample was shadow dried for 10 to 15 days after being rinsed with sterile distilled water. Then the dried sample was powdered and used for the biogenic synthesis (Anbuvannan et al., 2015).

2.2. Preparation of cassava peel extract

10% of extract was prepared by dissolving 10g of dried cassava peel powder in 100ml distilled water. The extract was incubated at 40°C for 24hrs in an orbital shaker at a speed of 60 – 70 rpm. Whatmann No.1 filter paper was used to filter the extract after the incubation and used for the proposed study (Hassan Basri et al., 2020).

2.3. Synthesising of Zinc oxide nanoparticle

ZnO nanoparticles were prepared by mixing 20 ml of cassava peel extract with 0.1M zinc acetate (MW-297.48) and kept for continues stirring in magnetic stirrer for 5hrs and later in a room temperature for 24hrs. 0.1M Zinc Acetate is used for synthesizing the nanoparticles because it is less toxic and easy to produce. After incubation, pale yellow coloured precipitation was centrifuged and used for further study (Anjali et al., 2022). All the chemicals used in the present study were AR grade and obtained from Hi media, India.

2.4. Characterisation of nanoparticle

2.4.1. UV-Visible spectroscopy

The synthesised zinc oxide nanoparticle was examined under UV Visible Spectrophotometer. After setting the baseline, the ZnO was scanned with a UV-Visible spectrophotometer (Labtronics LT291) at wavelengths ranging from 200 to 600 nm. The characteristic peaks were detected by keeping distilled water as a blank solution (Imade et al., 2022).

2.4.2. Fourier transform infrared spectroscopy (FTIR)

Fourier Transform Infra Red study was done for the identification of the functional group, molecular composition and structure present in the zinc oxide nanoparticles. For mid-range Infrared, scanning was done by keeping the wave number from 4000cm^{-1} to 400cm^{-1} (Shimadzu instrument) and finalised the compound in the extract (Yu et al., 2019).

2.4.3. X-ray diffraction (XRD)

The sample was analyzed using PAN analytical X-Pert PRO instrument. To determine the chemical composition and crystal structure of a synthesised nanomaterials X-Ray diffraction analysis was carried out (Imade et al., 2022).

2.4.4. Field emission scanning electron microscopy (FESEM)

Morphology and crystallite size were examined by Scanning Electron Microscope (SEM). The synthesised sample was centrifuged at 8000rpm for 10 minutes and fine powder was obtained by drying the pellet under hot plate (Awwad et al., 2019).

2.4.5. Zeta potential

The surface charge and the stability of the ZnO-NPs were measured using Malvern Zetasizer ZS (Raj and Lawerence, 2018).

2.4.6. Antibacterial activity

In order to evaluate the antibacterial study of the sample, Mueller-Hinton Agar (MHA) plates were used for well diffusion method. The zones of inhibitions were reported in millimetre (mm). For finding the antibacterial activity, overnight cultures of P. fluorescens, E.coli, K.pneumoniae, S.aureus and S. typhi were used as references (Raj and Lawerence, 2018).

3. Results and discussions

3.1. Preparation of extract

An aqueous extract of *Manihot esculenta* (Cassava) Peel, family Euphorbiaceae was used as a reducing and stabilizing agent for the synthesis of Zinc Oxide nanoparticles. The formation of pale yellow precipitate has confirmed the ZnO-NPs as shown in Figures 1 and 2.

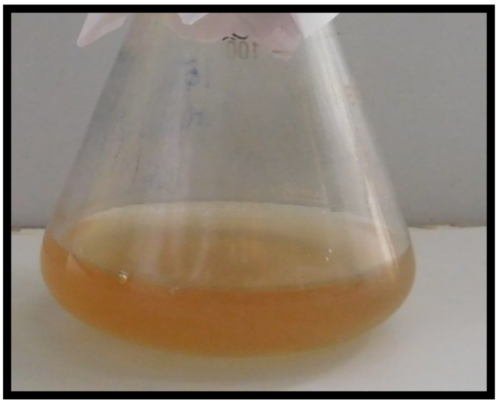


Figure 1. Biogenic synthesis of zinc oxide nanoparticle

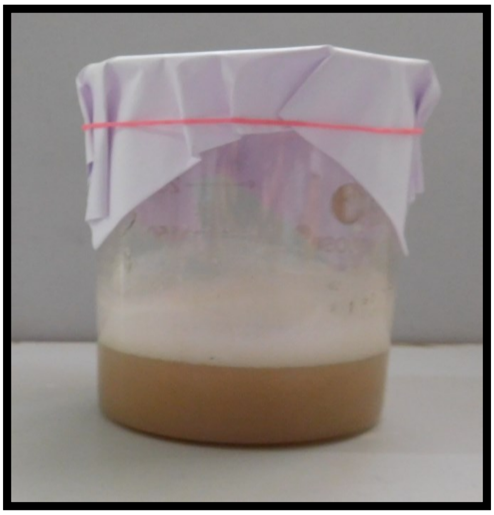


Figure 2. Formation of ZnO nanoparticles

3.2. Characterisation

3.2.1. UV-Visible spectroscopy analysis

For the sake of controlling the size and morphology, various plant phytochemicals were used by the researchers for synthesizing the nanoparticles. The reduction of metal ions to metal nanoparticles was happened by the presence of phytochemicals in the plant extract. The reduction reaction process was monitored by UV-Vis spectroscopic analysis. The reduction reaction in the UV Visible study is done by the presence of secondary metabolites present in the peel. The thumb rule states that the adsorption peak is ranged from 300 to 400nm for green synthesised ZnO-NPs (Barzinjy and Azeez, 2018). In the proposed study, the plasmon peak was observed at 380nm and it corroborated an efficient and successful green synthesis of ZnO-NPs. As stated clearly in the literatures, it was concluded that the purity of the extract was found after numerous processes. According to the theory of Mei, if a single sharp absorbance curve is found, the nanoparticles shape is spherical (Ezealisiji et al., 2019). However, from the above-mentioned study, it was confirmed that the produced zinc oxide nanoparticles were green synthesized and spherical in shape.

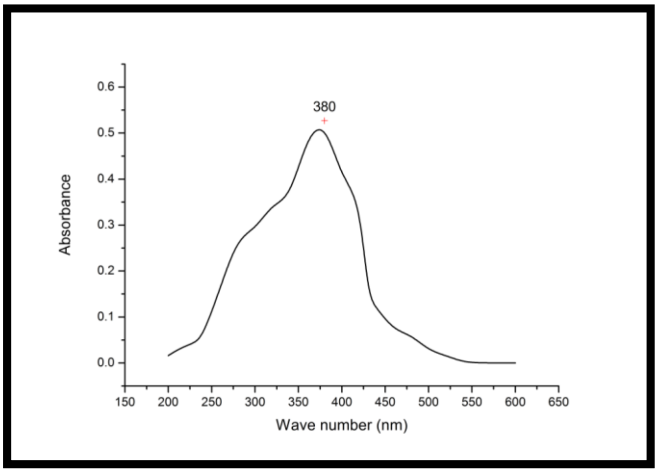


Figure 3. Graph showing the UV-Visible Spectroscopic analysis

3.2.2. Fourier transform infrared spectroscopy analysis (FTIR)

FTIR analysis was carried out to examine the structure of the compounds in the green synthesised zinc oxide nanoparticles. Specifically, FTIR spectrum of the ZnO-NPS has contained the peaks at 3356 cm^{-1}, 2978 cm^{-1}, 2391 cm^{-1}, 2306 cm^{-1}, 1635 cm^{-1}, 1550 cm^{-1}, 1381 cm^{-1}, 1165 cm^{-1}, 671 cm^{-1}, 601 cm^{-1}, 470 cm^{-1} and 432 cm^{-1} as shown in the Figure 3. The wide peak at 3356 cm^{-1} could be attributed to C-H stretching mode, the peak at 2978 cm^{-1} shows O-H stretching mode and the transitions to the C≡N stretching vibrations were found at 2306 cm^{-1} (Figure 4).

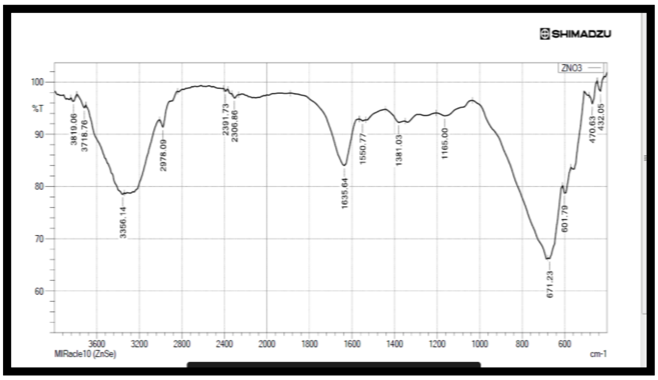


Figure 4. Graph of FTIR analysis

The peak at 1635 cm^{-1} shows C=O stretching mode of alkene group with two aromatic rings and the peak at 1550 cm^{-1}shows C=C stretching vibrations with aromatic ring and N-H bending alcohol, ether in secondary amine. The peak at 1381 cm^{-1} shows C-O-H bending mode and the peak at 1086 cm^{-1}shows the presence of C-O stretching vibration and peaks at 671 cm^{-1},640 cm^{-1},609 cm^{-1} and 578 cm^{-1} show C-Cl stretching vibrations. So it is obviously inferred that the samples have absorption peaks in the range of 3356.14 cm^{-1}, 2978.09 cm^{-1}, 1550.77 cm^{-1}, 1381.03 cm^{-1}, 1165.00 cm^{-1}, 671.23 cm^{-1} and 432.05 cm^{-1}. This shows that biological molecules are able to create and stabilise ZnO-NPs in the aqueous media simultaneously. Since the FTIR of Cassava peel extract has all the functional groups, they play an important role in the synthesis of ZnO-NPs (Sagar Raut and Thorat, 2015).

3.2.3. X-Ray diffraction analysis

The green synthesised ZnO-NPs were further studied using X-ray diffractrometer and the proposed study was carried out to analyze the surface morphology, size and crystalline nature of ZnO-NPs. The peaks obtained from the XRD pattern were sharp; the material is crystalline in nature. For XRD studies, a sample was made by applying a small quantity of sample on a glass plate. The start position of the scanning was at 10° and the end position of the scanning was at 80° and to find out the crystalline size of the green synthesised nanoparticles, the Scherrer's equation was used as mentioned in equation (1).

$$Dp = \frac{0.9\lambda}{\beta\cos\theta} \quad (1)$$

where Dp = crystallite size, λ = wavelength (1.5406 Å for Cu Kα), β = Line Broadening (radians) which is the Full-Width at Half-Maximum (FWHM) of the peak and θ = diffraction angle in radians. The strong peaks were identified at 28.6762°, 28.2663°, 32.6655°, 33.1234°, 39.0734°, 40.4977°, 46.0696°, 57.4133°, and 77.4292° (Figure 5) and it confirmed the structural properties of the material. From Table 1, the average size of the crystallite was found out (Ramesh et al., 2015).

Table 1 Geometric parameters of ZnO-NPs

2θ in °	FWHM in β	d-spacing in Å	Cos θ	Crystallite Size in nm
28.6762	0.1673	3.22212	0.9688	51.21
28.2663	0.0836	3.15511	0.9697	102.39
32.6655	0.0669	2.82744	0.9596	129.30
33.1234	0.1506	2.78646	0.9585	57.50
39.0734	0.3346	2.36358	0.9424	26.32
40.4977	0.0669	2.23016	0.9382	132.25
46.0696	0.2007	1.96984	0.9203	44.94
57.4133	0.2676	1.60759	0.8771	35.34
77.4292	0.4015	1.23263	0.7803	26.50
			Average	**67.31**

3.2.4. Field Emission Scanning Electron Microscopy analysis

FESEM (TESCAN MIRA) was used to measure the size and surface morphology of ZnO-NPs. FESEM images were taken to examine the shape and size of the synthesised zinc oxide nanoparticle. The exterior morphology confirmed the structure of the nanoparticles in their agglomerated form. The particles were found to be mostly horizontal in shape, which was further confirmed using XRD. Round spherical structure with 50 to 94 nm size were observed as shown in Figure 6. Therefore, it was observed that the particle size obtained from the XRD analysis coincides with the FESEM result.

3.2.5. Zeta potential analysis

Zeta potential of the ZnO-NPs was investigated using a malvern zeta sizer. Zeta potential (z) is a typical measurement of the surface charge and defines colloidal stability of the particles. It was suggested (Modena et al., 2019) that the suspensions which show ≥ 15 mV were termed as incipient stability. The zeta potential of synthesized ZnO-NPs in distilled water was recorded as 25.9 mV and can be considered as stable colloidal solution. The particles are positively charged and they are moderately dispersed in the medium (Figure 7). The result shows a good sign that these synthesised ZnO-NPS have appreciable adsorption sites for further degradation processes.

3.2.6. Anti bacterial activity

The anti bacterial activity was carried out using four bacterial pathogens. Among the five bacteria tried, the ZnO-NPs were more active against all the pathogens using agar well diffusion method as shown in Table 2. The ZnO-NPs led to destructions of bacterial cell wall and destroyed the bacteria. Moreover, the diameter of the Zone of Inhibition (ZOI) varied for organisms, so the zone of inhibition was measured to find the growth of the microorganisms. The MHA agar plates were prepared by dissolving 39.8g in 1000 ml and it was autoclaved at 121^0C for 15 minutes. Autoclaving media was poured to petri plate and allowed for solidification (Figures 8 and 9).

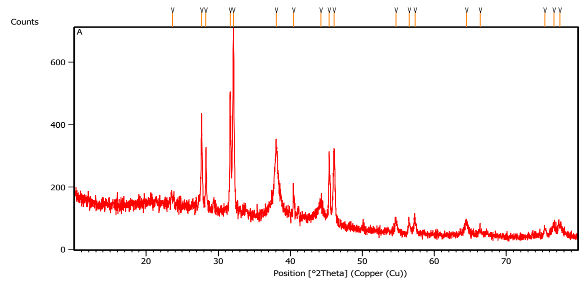


Figure 5. Graph showing XRD analysis

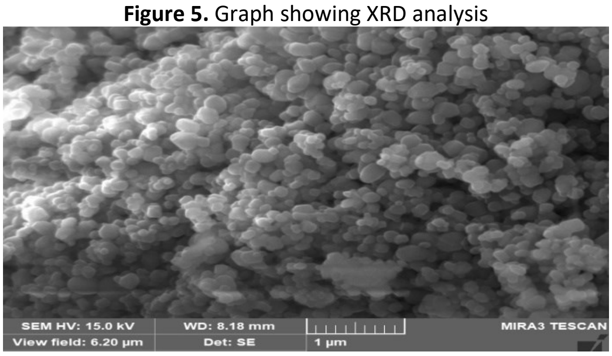


Figure 6. Graph of FESEM analysis

Table 2. Anti bacterial activity of biosynthesised ZnO-NPs.

Sample Concentration in µl	Zone of inhibition in mm				
	Pseudomonas fluorescens (1)	Escherichia coli (2)	Salmonella typhi (3)	Staphylococcus Aureus (4)	Klebsiella pneumonia (5)
4	0	2	2	2	2
8	2	4	4	3	3
DMSO	0	0	0	0	0
Disc	0	8	9	5	6

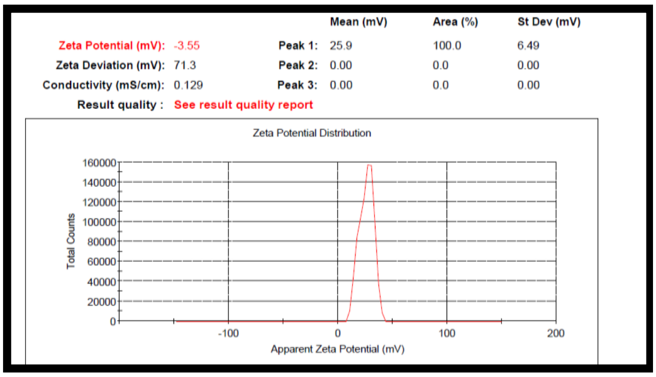


Figure 7. Graph of Zeta Potential analysis

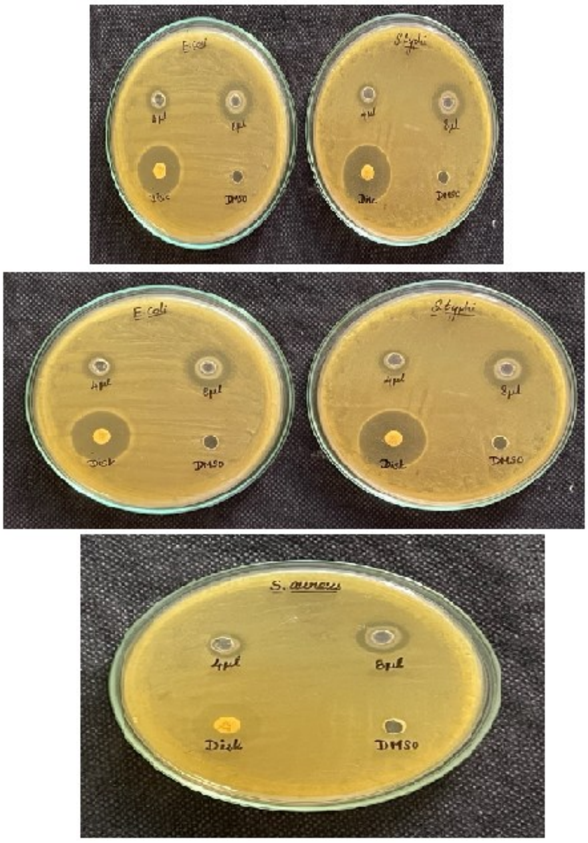


Figure 8. *Images of Antimicrobial activity of biosynthesised ZnO-NPs*

The bacterial strain plates were inoculated under aseptic conditions and wells were made with crock borer of diameter 5mm, 10 mm and 20 mm and ZnO-NPs were added and incubated at 37 °C for 24 hours. The bacterial growth was measured by measuring the diameter of the inhibition zones after the incubation period. The culture used for this study was 18 to 24 hrs single colonies on agar plates that were used to prepare the bacterial suspension with the turbidity of 0.5 McFarland (equal to 1.5×108 colony-forming units (CFU)/ml). Bacterial suspension Turbidity was measured at 600 nm (Haseena et al., 2022).

The antibacterial study was conducted for both gram positive and gram-negative bacteria namely Staphylococcus aureus, Pseudomonas fluorescens, Escherichia coli, Salmonella typhi, Klebsiella pneumonia. In order to measure the diameter of the bacterial inhibition zone, Well Diffusion method was employed for testing the inhibition zone of the bacteria, along with gram-positive and gram-negative bacteria, positive control as Ceftazidime and negative control as Dimethyl sulfoxide were also used (Sampathkumar et al., 2022). Well Diffusion method is the widely used method to evaluate the antimicrobial activity of plants and microbial extracts.

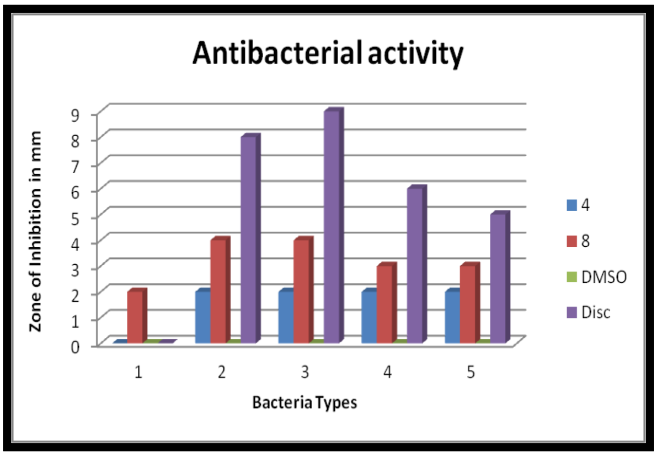


Figure 9. Inhibition zones comparison chart

From the graph, it is clear that the zone of inhibitions gets increased with the increase in the concentration of ZnO Nanoparticles which in turn, makes the higher concentrations of synthesised nanoparticles to have a better antibacterial activity. Also the gram positive bacteria namely Escherichia Coli and Salmonella Typhi have showed better growth comparing to the other gram-positive and gram-negative bacteria (Ragunathan et al., 2022; Kandasamy et al., 2022).

4. Conclusion

The zinc oxide nanoparticles were successfully synthesised with the help of *Manihot esculenta* **(**cassava peel) which is a waste material. In addition to that, the bio synthesised nanoparticles were characterized using UV-Vis Spectroscopy and the Plasmon peak was observed at

380nm, which had shown the presence of ZnO nanoparticles and the same time, it was found out that the shape of the particle was spherical. Moreover, the presence of functional group and molecular structure pertinent to the stability of the nanoparticles were confirmed by FTIR analysis. The average crystallite size was recorded to be 67.31nm through XRD analysis and simultaneously, it was proved that the particles were in nano-range. Likewise, the shape of the particle was determined as round spherical from FESEM analysis with the size ranged from 50 to 94 nm. So, the synthesised nanoparticles had shown better performance against both gram positive and gram-negative bacteria. Thus, from the study, it is proven that the biosynthesised nanoparticles using agro based waste materials are acknowledged to be the best for its eco-friendly products when compared to the materials produced using chemicals. Also, after further clinical study and cell line study, the Zinc oxide nanoparticles as an alternative drug instead of commercial antibiotics.

Author contributions

N.Jothi Lakshmi: Identified the problem and found out the suitable material for the study, performed various tests and analysed to check the suitability of the material and involved in manuscript preparation. S.Anandakumar: Performed UV-Visible spectroscopy analysis and interpreted the results, involved in sample preparation and verification of manuscript. V. Sampathkumar: Supported the study with historical data and has been major stand towards the completion of the manuscript. All authors read and approved the final manuscript.

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